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THE GENESIS OF THE ORGANIZATION OF THE INSECT EGG¹

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I. THE COMPLEXITY OF ORGANIZATION OF THE INSECT EGG

1. *Introduction*

THE morphological and experimental investigations of the germ-cell cycle in insects which the writer has carried on during the past ten years have resulted in the accumulation of many data which indicate the complexity of organization of the eggs of these animals, and suggest hypotheses regarding the nature and genesis of this organization. That the animal egg at the time development begins does not consist of a homogeneous mass of protoplasm, as the old theory of epigenesis required, but is a highly organized cell containing various kinds of protoplasm localized in definite regions has been proved conclusively by numerous investigators working with the eggs of many different species. The degree of organization at the time of fertilization varies according to the species of animal, but all embryologists admit that the insect egg is one of the most highly organized of all.

The nature and genesis of the different kinds of protoplasm in the insect egg is the principal problem discussed in the following pages. This problem is the logical successor of those dealing with cell lineage and the organization of the egg at the time development begins. The re-

¹ Presented to the Johns Hopkins Scientific Association on October 9, 1917.

lations between this problem and the larger problem of heredity and development are very close indeed. The study of heredity is concerned not only with the adult animal, but also with every stage in the development of the adult since the fertilized egg, or embryo, or larva that arises from it, is an individual just as is the fully developed animal. The organization of the fertilized egg is the result of the processes of differentiation that take place at each stage in the history of the egg and sperm from the time the primordial germ cells are segregated until the highly specialized gametes have become fully formed. The period extending from the formation of the primordial germ cells to and through the growth period of the gametes is one of the least known in the entire history of the individual. It is nevertheless a most important period, for during this time, at least in the insect egg, the principal axes of the individual are established and different kinds of cytoplasm are elaborated and localized that are predetermined to form definite parts of the embryo.

The stages in the germ-cell cycle in insects belonging to different orders, families, genera or species are often quite different, as is to be expected, hence the data on which this paper is based were derived from the study of a number of species. On this account it seems best to give an abridged description of the germ-cell cycle in one group and include, wherever necessary, data derived from the study of other groups. The principal work has been carried on with representatives of the orders Diptera, Hymenoptera and Coleoptera, and of these, the order Coleoptera has furnished the best material for experimental purposes. We will select, therefore, the eggs of certain chrysomelid beetles for descriptive purposes.

2. *The Structure of the Insect Egg at the Time of Deposition*¹

At the time of deposition (Fig. 1) the beetle's egg consists largely of deutoplasm—a substance which is used

¹ Hegner, 1909, *Journ. Exp. Zool.*, Vol. 6.

up during the growth of the embryo. This deutoplasm is composed of vitelline spheres, which contain refringent granules, the vitelline bodies, and of oil globules. The deutoplasmic bodies are embedded in a viscid cytoplasmic matrix which, however, is very slight in amount as compared with the deutoplasmic material. At the periphery of the egg is a thin cortical layer of cytoplasm which is continuous with the cytoplasm in which the vitelline spheres lie. A short distance back of the anterior pole of the egg is a thickening of this cortical layer in which the maturation divisions of the oocyte nucleus occur. The cytoplasm appears to be homogeneous except at the posterior end, at which place, in many insects, inclusions have been discovered which appear to play a rôle in the formation of the primordial germ cells.²

These inclusions in the chrysomelid beetles take the form of a polar disc of granules which I have called germ-cell, keimbahn, or germ-line determinants.³ Similar inclusions (Fig. 2) have been

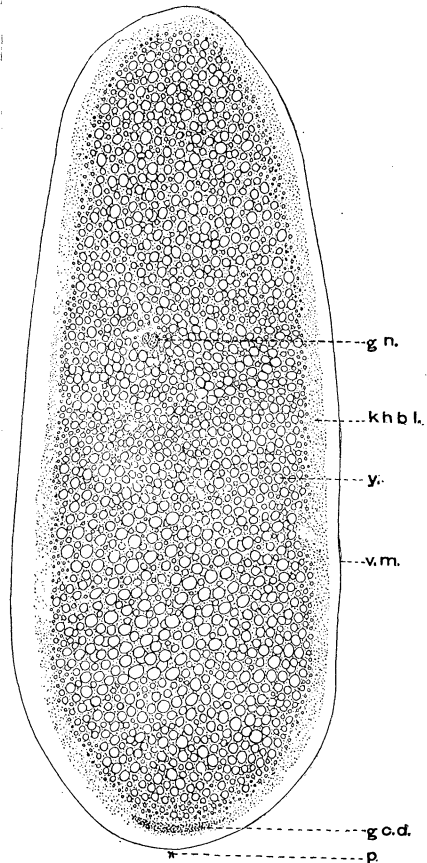


FIG. 1. Longitudinal section through an egg of *Calligrapha bigsbyana* four hours after deposition. *gcd* = germ-cell determinants. *gn* = germ-nuclei copulating. *khbl* = cortical layer of cytoplasm. *p* = posterior end. *vm* = vitelline membrane. *y* = yolk. (From Hegner, 1909.)

² Hegner, 1909, *Journ. Morph.*, Vol. 20.

³ Hegner, 1908, *Biol. Bull.*, Vol. 16.

⁴ Weisman, 1863, *Zeit. f. wiss. Zool.*, Bd. 13; Metschnikoff, 1866, *ibid.*,

noted in Diptera,⁴ in parasitic Hymenoptera,⁵ and in Hymenopterous gall flies.⁶ There should also be mentioned in this connection minute bodies that have been

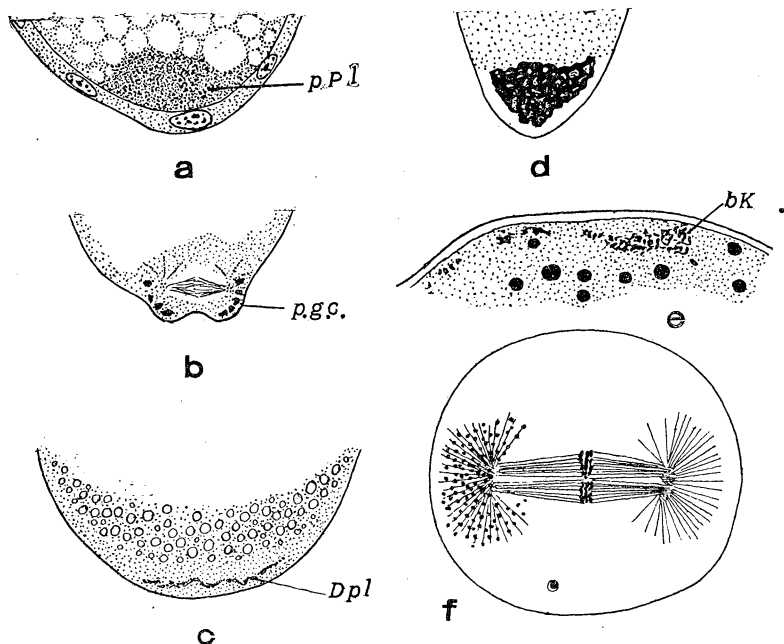


FIG. 2. Germ-line determinants in the eggs of various animals. *a.* Poleplasm (*pPl*) at the posterior end of the egg of *Miastor* (Hegner, 1914). *b.* Keimbahnplasma (*pgc*) at the posterior end of the egg of *Chironomus*. One of the four cleavage nuclei in undergoing mitosis. (Hasper, 1911.) *c.* Dotterplatte (*Dpl*) at the posterior end of the egg of *Calliphora*. (Noack, 1901.) *d.* Germ-line determinant at the posterior end of the egg of *Apanteles*. (Hegner, 1915.) *e.* Besondere Körper (*bK*) in the egg of *Sagitta*. (Elpatiewsky, 1909.) *f.* Ectosomen at one end of the first cleavage spindle in the egg of *Cyclops*. (Amma, 1911.)

discovered in the cytoplasm of the eggs of the carpenter ant, *Camponotus*, and in those of various other insects.⁷

Bd. 16; Ritter, 1890, *ibid.*, Bd. 50; Noack, 1901, *ibid.*, Bd. 70; Kahle, 1908, *Zoologica*, Bd. 21; Hasper, 1911, *Zool. Jahrb.*, Bd. 31; Hegner, 1912, *Science*, Vol. 36; Hegner, 1914, *Journ. Morph.*, Vol. 25.

⁵ Silvestri, 1906, *Boll. Labor. Zool. R. Sc. Agr. Portici*, Vol. 1; Silvestri, 1908, *ibid.*, Vol. 3; Silvestri, 1914, *Anat. Anz.*, Bd. 47; Silvestri, 1915, *Boll. Labor. Zool. R. Sc. Agr. Portici*, Vol. 10; Silvestri, 1916, *Rend. D. R. Accad. D. Lincei*, Vol. 25; Martin, 1914, *Zeit. f. wiss. Zool.*, Bd. 110; Hegner, 1914, *Anat. Anz.*, Bd. 46; Hegner, 1915, *Journ. Morph.*, Vol. 26.

⁶ Hegner, 1915, *Journ. Morph.*, Vol. 26.

⁷ Blochmann, 1886, *Festsch. nat.-med. Verein zu Heidelberg*; Sule, 1906,

These have been considered symbiotic bacteria, but their true nature remains yet to be definitely established.

3. Cleavage

The first cleavage nucleus of the beetle's egg lies somewhat anterior of the center in a small island of cytoplasm that is continuous with the cytoplasm that surrounds the deutoplasmic bodies (Fig. 1, *gn*). During early cleavage no cell walls are formed, but after each division the daughter nuclei move a short distance apart and then divide again. Successive divisions and migrations of the cleavage nuclei (Fig. 3) finally result in the production of

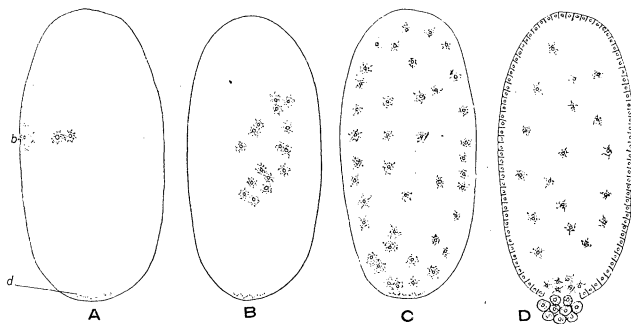


FIG. 3. Diagrams showing four stages in the cleavage and blastoderm formation of the chrysomelid beetle's egg. *a*. Two-cell stage. *b* = polar bodies. *d* = pole disc. *b*. Sixteen-cell stage. *c*. Just before formation of blastoderm. *d*. Blastoderm stage. At the posterior end are the primordial germ cells. (Hegner, 1909, 1914.)

hundreds of nuclei, which come to lie just beneath the cortical layer of cytoplasm, and are each surrounded by an irregular mass of cytoplasm. The fusion of these cytoplasmic masses with the cortical layer then takes place, followed by the intervention of cell walls, thus forming a blastoderm of a single layer of cells, each of which contains a cleavage nucleus, part of the cytoplasm which it brought to the periphery with it, and a portion of the

Stzber. böhm. Gesellsch. Wiss. Prag.; Sulc, 1910, *ibid.*; Merceir, 1907, *Arch. Protistenk.*, Bd. 9; Pierantoni, 1910, *Zool. Anz.*, Bd. 36; Buchner, 1912, *Arch. Protistenk.*, Bd. 26; Tanguary, 1913, *Bull. Ill. St. Lab. Nat. Hist.*, Vol. 9; Hegner, 1915, *Journ. Morph.*, Vol. 26.

cortical layer. Not all of the cleavage nuclei take part in blastoderm formation, many of them remaining behind in the yolk to aid in breaking down this substance.

4. *The Origin of the Primordial Germ Cells*

Blastoderm formation is interrupted at the posterior end of certain chrysomelid beetles' eggs by the segregation of the primordial germ cells (Fig. 4). Those cleav-

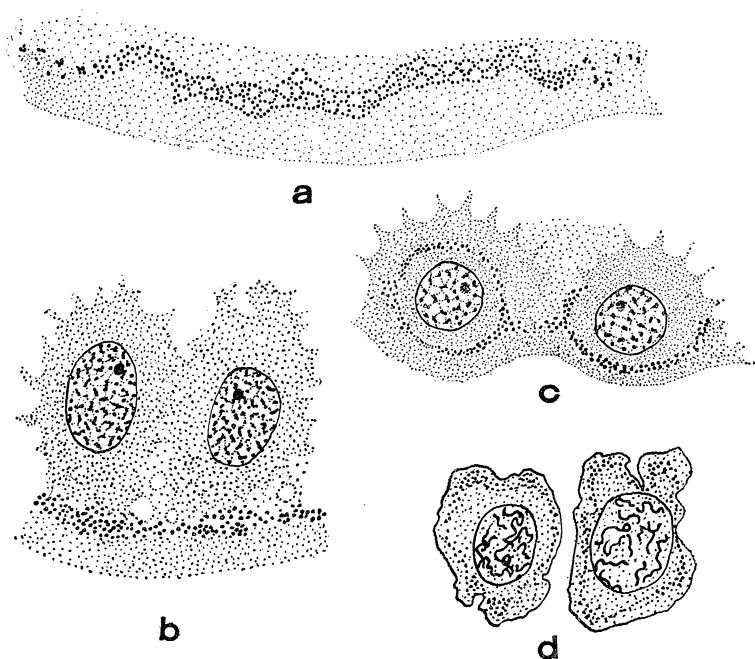


FIG. 4. Portions of the posterior end of eggs of *Calligrapha* showing stages in the formation of the primordial germ cells. *a*. Pole-disc granules in the cortical layer. *b*. Two cleavage nuclei just before the pole disc is reached. *c*. Two cleavage nuclei becoming surrounded by pole disc granules. *d*. Two primordial germ cells entirely separated from the egg. (Hegner, 1909.)

age nuclei that encounter the granules of the pole-disc do not produce blastoderm cells, but continue their migration and are finally cut off from the rest of the egg as distinct cells. These are the primordial germ cells, of which there are sixteen. Each of these cells contains a portion of the cortical layer that includes pole-disc gran-

ules, thus differing in content from the blastoderm cells. They differ also from blastoderm cells in size, being considerably larger. This is probably due at first to the inclusion of the pole-disc granules, but later a greater difference in size is brought about by the failure of the germ cells to divide as rapidly as do the blastoderm cells.

An early origin of the primordial germ cells in a similar manner has been described in a number of insects besides Coleoptera, especially in the midge, *Chironomus*,⁸ where these cells are derived from one of the cleavage nuclei at the four-cell stage; in the pedogenetic larva of the fly, *Miastor*,⁹ where one of the first eight cleavage nuclei becomes the nucleus of the primordial germ cell, in the fly, *Calliphora*,¹⁰ and in parasitic Hymenoptera.¹¹

5. The Formation of the Ovaries

In chrysomelid beetles, *Chironomus*, *Miastor* and certain other species of insects, the primordial germ cells undergo a multiplication period shortly after they are formed. This is followed by a period during which they become lodged within the embryo—either by the shifting of the surrounding tissues or by migration or by both these processes. At this time also they become separated into two groups; in chrysomelid beetles each group appears to contain thirty-two germ cells; in *Miastor* each consists of four germ cells. One group becomes located on either side of the embryo and later gives rise to one half of the germ glands. The sex of the individual can be determined by the morphology of the germ glands before the young hatches.

The further history of the germ cells in female insects is in general as follows. From each primitive ovary a number of ovarian tubules arise each containing many germ cells (oogonia) which have undergone a multiplication period. The oogonia finally cease dividing and the

⁸ Hasper, 1911, *Zool. Jahrb.*, Bd. 31.

⁹ Kahle, 1908, *Zoologica*, Bd. 21; Hegner, 1914, *Journ. Morph.*, Vol. 25.

¹⁰ Noack, 1901, *Zeit. f. wiss. Zool.*, Bd. 70.

¹¹ Silvestri, *l. c.*

ultimate oogonia are ready to enter upon the growth period. A period of differentiation may or may not intervene, according to the species, during which nurse cells are formed. When the oocytes have reached their full size they separate from the ovarian tubule, pass down the oviduct into the vagina and are deposited. Each egg is surrounded by two membranes; a thin inner vitelline membrane and a thicker, outer membrane, the chorion.

6. *The Complexity of Organization of the Insect Egg.*

(a) *Comparison between Eggs of Insects and Those of Other Animals.*—Insect eggs differ greatly from those usually employed for the study of egg organization, since they are, as a rule, laid in the air and not in the water, and because cleavage is of the superficial type, cell walls being absent until a comparatively late cleavage stage. The eggs of chrysomelid beetles are particularly favorable for study, since they may be subjected to the most violent experimental conditions without preventing their development.¹²

In insect eggs the character of the blastoderm cells depends, as in holoblastic eggs, upon the kinds of protoplasm they contain, but all those phenomena connected with the position of the cleavage spindle, which have been so carefully studied in the eggs of mollusks, worms, ascidians and other animals, can have no influence upon the localization of different substances in various parts of an insect egg, because in the latter the volume of the egg is thousands of times greater than that of the cleavage spindle. Furthermore in holoblastic eggs differentiated substances are segregated in different cells during early cleavage and are there isolated by cell walls, and to this isolation is attributed in large part the progress of differentiation; but in the insect egg the different kinds of cytoplasm are in direct continuity until hundreds of cleavage nuclei are present, and are not separated by cell walls until the blastoderm is fully formed.

¹² Hegner, 1908, *Biol. Bull.*, Vol. 16; 1909, *Journ. Exp. Zool.*, Vol. 6; 1911, *Biol. Bull.*, Vol. 20.

(b) *Results of Experiments with Gravity and Centrifugal Force.*—A chrysomelid beetle, such as *Calligrapha* or *Leptinotarsa*, during the process of egg-laying clings to the under side of a leaf and the end of the egg that emerges first is glued to the leaf by a viscid secretion. Then the egg is pushed back away from the abdomen and another is laid¹³ (Fig. 5). In this way from four to

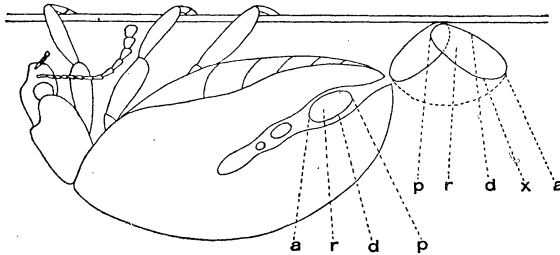


FIG. 5. Diagram showing a chrysomelid beetle, *Calligrapha bigsbyana*, clinging to the underside of a willow leaf and laying her eggs. The relation between the orientation of the egg before and after deposition is indicated by the letters. *a* = anterior. *d* = dorsal. *l* = left. *p* = posterior. *r* = right. *x* = anterior ventral surface where a spot of India ink was placed as a guide for orienting the eggs during experiments. (Hegner, 1909.)

eighty eggs are laid in one group within a period of about an hour. These eggs hatch in approximately five days. A few hours before they hatch the young can be seen distinctly through the semi-transparent egg shell. An examination of hundreds of eggs at this stage in their development has established the fact that the posterior end of the egg is attached to the leaf and the anterior end is free. In every other respect the orientation of the young in the egg corresponds to that of the egg as it lay within the body of the mother before deposition; that is, the ends and various surfaces of the egg are definitely determined before deposition and correspond to the orientation of the mother as indicated in the diagram (Fig. 5). This rigid correspondence between the orientation of the egg and that of the adult is known as the "law of orientation" which was first discovered by Hallez in 1886.

Since the eggs of these beetles are usually attached to

¹³ Hegner, 1909, *Journ. Exp. Zool.*, Vol. 6.

the underside of leaves, it was suggested that the orientation of the young might depend upon the force of gravity, but eggs that were first marked with India ink and then placed in every conceivable position with respect to gravity proceeded to develop as though undisturbed.¹⁴ It seemed from this, therefore, that the position of the young must be predetermined in the undeveloped egg.

Several kinds of experiments were performed in order to discover the complexity and fixity of this apparent organization. First, the eggs were subjected to a force

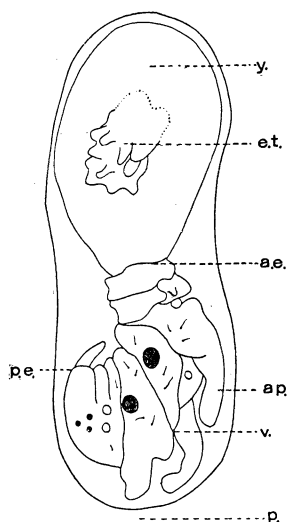


FIG. 6. Side view of a freshly laid egg of *Calligrapha multipunctata*, which was centrifuged for sixteen hours and then taken out and allowed to develop for nine days. *ae* = anterior end of embryo. *ap* = appendage of thorax. *et* = embryonic tissue. *p* = posterior end of egg. *pe* = posterior end of embryo. *v* = ventral. *y* = yolk. (Hegner, 1909.)

greater than gravity by means of a centrifugal machine. Hundreds of eggs were revolved at different rates of speed for various lengths of time and in many different positions. A description of one experiment will serve to illustrate the results obtained.¹⁵ In this experiment freshly laid eggs were placed in cavities in a block of paraffin with the posterior end toward the center of rotation, and were revolved in a hydraulic centrifuge for sixteen hours. The heavier substances were thrown to the outer end and the lighter protoplasm accumulated at the inner end, where an embryo developed (Fig. 6). It is perfectly evident that the protoplasm from the various parts of the egg has, in its new position, developed into the tissue that it would have given rise to if

it had been left undisturbed. Normally the yolk would be surrounded by the embryonic tissue and would be en-

¹⁴ Wheeler, 1889, *Journ. Morph.*, Vol. 3; Hegner, 1909, *Journ. Exp. Zool.*, Vol. 6.

¹⁵ Hegner, 1909, *Journ. Exp. Zool.*, Vol. 6.

closed by the mid-intestine, but in this case a dwarf embryo has developed without growing around the nutritive material.

The effects of centrifugal force upon insect eggs are different from those produced upon the other types of eggs that have been employed for such experiments. In the eggs of worms,¹⁶ mollusks,¹⁷ etc., apparently the materials that undergo stratification under the influence of centrifugal force have no influence upon the "ground substance" which is "the seat of polarity and pattern of organization of the cell." In the insect egg, the organized protoplasm is almost entirely limited to the cortical layer and this layer may be shifted away from the periphery by a sufficient force and may become massed at the inner light end when an undeveloped egg is centrifuged.

Since the cytoplasm develops in its new situation and proceeds to build up an embryo as nearly normal as is possible under the conditions imposed upon it, it is evident that the potencies of the cytoplasmic areas are predetermined at the time the egg is laid.

It was hoped by means of these experiments with centrifugal force to throw the pole-disc granules and the cytoplasm containing them into some other part of the egg. If the germ cells arose from this material in its new position the conclusion would have been convincing that these substances were necessary for the formation of the reproductive cells. Unfortunately, although the cortical layer at the posterior end was shifted by the centrifugal force, it was impossible to locate accurately the germ cells in the embryos that developed from the eggs that were thus operated upon.

(c) *Relation between Cleavage Nuclei and Egg Organization.*—During the course of my early studies of chrysomelid eggs it occurred to me that the nuclei that result from early cleavage might be definite in number and in

¹⁶ Lillie, F. R., 1906, *Journ. Exp. Zool.*, Vol. 3.

¹⁷ Conklin, 1917, *Journ. Exp. Zool.*, Vol. 22.

distribution and that they might be qualitatively different. If this were true, then nuclei of one sort might always migrate into one part of the egg and might determine the nature of the tissue that developed there, and nuclei of other sorts might likewise become located in other predetermined parts of the egg. Careful studies of the origin and migration of the cleavage nuclei¹⁸ have led to the conclusion that the distribution of these nuclei is adventitious and that they are all potentially alike—that is are totipotent—a view that is now held by most embryologists regarding the relative importance of nucleus and cytoplasm during cleavage. That the nuclei may play a part in the differentiation of the cortical layer of cytoplasm during the cleavage period is highly improbable, since definite cytoplasmic organization already exists before cleavage begins. The factors brought into the egg by the spermatozoon, however, have an opportunity at this time to modify the initial organization and thus the early embryo may exhibit paternal characteristics. Whether or not such an influence is exerted at this time is not known.

The kind of tissue that develops from any part of the egg, therefore, depends upon the kind of cytoplasm encountered by the nuclei.

(d) *Complexity of Organization as Indicated by the Development of Parts of Eggs.*—More convincing evidence of the presence of a complex and fixed organization in the cytoplasm was derived from operations performed upon eggs with a hot needle. Parts of the freshly deposited eggs were killed by being touched with a hot needle and these parts were thus prevented from taking part in development. The living portions of the eggs continued to develop and in every case produced those parts of the embryo that they would have formed if the egg had not been injured.¹⁹ This seems to prove that every part of the egg cytoplasm is set aside for the pro-

¹⁸ Hegner, 1914, *Journ. Morph.*, Vol. 25.

¹⁹ Hegner, 1911, *Biol. Bull.*, Vol. 20.

duction of a definite part of the embryo, and hence of the larva and adult, and that the cytoplasm is therefore highly organized at the time the egg is fertilized. After such experiments there is no regeneration of substances.

As stated above, the cortical layer of cytoplasm is visibly alike throughout except at the posterior end, where it has embedded in it the pole-disc granules. One of the most interesting results of the operations performed with the hot needle was obtained by killing the posterior portion of the egg containing the pole-disc (Fig. 7). Eggs

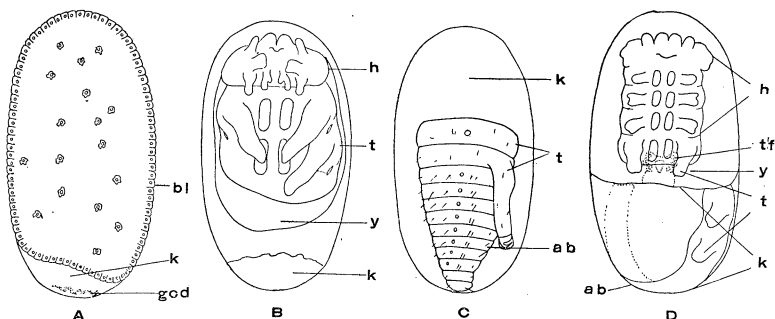


FIG. 7. Diagrams showing the results of killing parts of the eggs of *Leptinotarsa decemlineata* with a hot needle. (Hegner, 1911.)

a. Longitudinal section through an egg twenty-four hours old. The posterior end (*k*) was killed just after the egg was deposited (conditions as in Fig. 1). No germ cells were produced.

b. Ventral view of an embryo three days old. The posterior end (*k*) was killed just after the egg was deposited. The part that remained alive gave rise to the head and about half of the thorax.

c. Side view of an egg five days old. The anterior end (*k*) was killed in the blastoderm stage (Fig. 3, *d*). The part that remained alive produced the abdomen (*ab*) and about half of the thorax (*t*).

d. Ventral view of an egg three days old. The posterior end (*k*) was killed when the embryo was two days old. The anterior half continued to develop. The independence of the tissues is indicated by the minute end of the tail-fold (*tf*) which developed normally after being separated from the rest of the embryo. The two parts of the egg underwent a revolution of ninety degrees during the twenty-four hours succeeding the experiment.

ab = abdomen. *bl* = blastoderm. *gcd* = pole-disc. *h* = head. *k* = portion of egg killed with hot needle. *t* = thorax. *tf* = tail fold. *y* = yolk.

thus modified produced embryos without germ cells, proving that this cytoplasmic region is necessary for their formation. The castration of the individual may also be performed in a similar fashion after the germ cells have been extruded from the egg (Fig. 3, *d*), and it is interesting to note that sexless chicks have recently been pro-

duced in a similar fashion, by removing the region of the embryo from which the germ cells arise.²⁰

The blastoderm is of course definitely organized, since its cells contain organized cytoplasm, and by killing parts of the eggs in the blastoderm and later stages results were obtained similar to those produced when fresh eggs were operated upon (Fig. 7, *b, c, d*).

7. *Summary of Part I*

Summarizing the data briefly given above, we may say:

1. Morphological and experimental studies have proved that the eggs of animals are more or less highly organized at the time of fertilization.

2. We know almost nothing about the nature and genesis of this organization.

3. Descriptions are given of the condition of the eggs of certain chrysomelid beetles at the time of deposition, of the stages of cleavage and blastoderm formation, of the origin of the germ cells, and of the principal stages in the germ-cell cycle.

4. The eggs of certain chrysomelid beetles and of other insects are definitely organized when deposited as indicated by observations on normally developing eggs and by experiments with gravity.

5. This organization exists in the cytoplasm as indicated by a morphological study of cleavage, by experiments with gravity and centrifugal force, and by killing with a hot needle parts of eggs in various stages of development.

6. These observations and experiments prove also that the nuclei up to the time of blastoderm formation are totipotent.

II. THE GENESIS OF THE ORGANIZATION OF THE INSECT EGG

1. *Introduction.*

I have decided to consider the organization of the egg only in this discussion, since it contains everything neces-

²⁰ Reagan, 1916, Abstracts 14 annual meeting Amer. Soc. Zool.

sary for the production of a complete organism. In many species of insects and other animals, parthenogenesis is a normal phenomenon and in many species whose eggs must ordinarily be fertilized development may be initiated by artificial means.²¹ Among these are the eggs of the silkworm moth. It also seems certain that the cytoplasmic regions of the insect egg have reached a high state of morphological and physiological differentiation before fertilization, judging from the results outlined in Part I. of this paper. The cleavage nuclei may possibly exert an influence upon the cortical layer of cytoplasm before the blastoderm is formed, thereby enabling the paternal contribution to the zygote to act, but besides being very improbable, such a phenomenon would, of course, follow rather than precede the establishment of the advanced state of organization that exists in the undeveloped egg.

2. *Constitution of the Primordial Germ Cells*

In certain beetles, flies and parasitic Hymenoptera, the primordial germ cells are visibly different from the rest of the embryonic cells that arise at about the same time. This difference is primarily due to the inclusion within them of visible substances that are located in the egg material from which they originate. The eggs of these different insects are similar in certain respects and different in others. In every instance, however, this visible substance, which forms the germ-line determinants, is situated near the posterior end of the egg, and it is at this point that the primordial germ cells are formed. The origin of the germ-line determinants is not definitely known in any insect, but their position in the egg and their granular appearance are constant.

It has often been pointed out that the primordial germ cells remain in a comparatively undifferentiated state until the individual in which they lie has almost reached maturity, and that they then undergo changes during

²¹ Loeb, 1913, "Artificial Parthenogenesis and Fertilization," Chicago.

which they reach a high state of specialization. The discovery of axial gradients of metabolism in the eggs of certain animals in an anterior-posterior direction²² suggests that this may also be true of insect eggs. If such gradients exist in insect eggs and if the metabolic activity decreases gradually from the anterior to the posterior end, then the primordial germ cells, which arise at the extreme posterior end, are actually the least active metabolically of all the cells of the embryo. Their early separation from the egg would also tend to keep them in an undifferentiated condition since they are on this account less likely to be influenced by the rest of the embryo.

The primordial germ cells in these insect eggs are thus visibly different because of the presence of germ-line determinants and are probably physiologically different, at least in part, because of their position at the posterior end of the egg.

The contents of these cells are as follows (Fig. 4): (1) part of the cortical layer of cytoplasm, (2) part of the cytoplasm which surrounds the cleavage nuclei and which is collected from among the yolk globules, (3) part of the germ-line determinants, and (4) a nucleus with the full amount of chromatin. The fourth item is mentioned because in *Miastor* all of the nuclei that form somatic cells undergo a diminution process, being similar in this respect to *Ascaris*. This chromatin is in *Miastor* entirely maternal since the eggs of this fly that have been studied, develop parthenogenetically.

Nothing very definite has been discovered regarding the arrangement of these substances in the germ cells. The nucleus lies near the center in all of them; the two kinds of cytoplasm soon become indistinguishable; and the germ-line determinants may, at first, be more or less evenly distributed throughout the cytoplasm, as in chrysomelid beetles and *Miastor*, or may be clumped in various parts of the cell, as in *Chironomus*. In every case, however, the germ-line determinants evidently become

²² Child, 1916, *Biol. Bull.*, Vol. 30.

more or less evenly scattered since they cannot be distinguished in later stages in the germ-cell cycle.

3. Differential Divisions during the Formation of Nurse Cells

There is no evidence of any definite localization of substances or physiological processes in the primordial germ cells when formed, nor do these cells exhibit recognizable polarity or symmetry of any kind. As described in preceding pages, they multiply; migrate into or are enveloped by the tissues of the embryo; separate into two groups from which the ovaries on either side of the body arise; and then pass through another period of multiplication. This brings them to the stage just preceding the growth period. At this time phenomena occur in the ovaries of certain species of insects that have a direct bearing upon

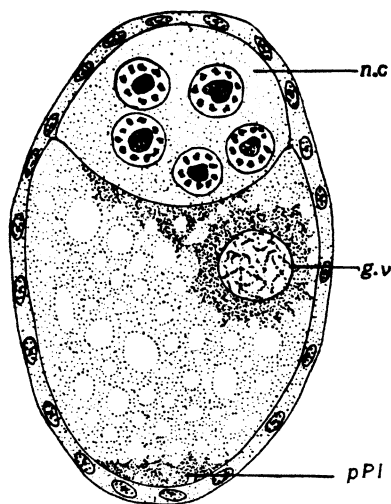


FIG. 8. Longitudinal section through a young oocyte of *Miastor* and its accompanying nurse cells (nc). gv = germinal vesicle. (Hegner, 1914.)

our problem; these are concerned principally with the differentiation of oocytes and nurse cells. In *Miastor* the nurse cells are mesodermal in origin, and a group of nurse cells and one oocyte become enclosed within a sheath of epithelial cells (Fig. 8). As the oocyte increases in size it elongates, and then for the first time in its history exhibits recognizable polarity; the anterior end adjoining the group of nurse cells. Polarity may, however, have been present from the time the primordial germ cell was first formed, corresponding to that of the parental egg. The germinal vesicle soon becomes eccentric, but whether or not this indicates that bilateral symmetry has also been

determined, as it does in certain other insects, is unknown. It is thus certain that polarity exists soon after the beginning of the growth period and that bilaterality is probably also established at an early stage.

The differentiation of oocytes and nurse cells in dytiscid and gyrinid beetles is of peculiar interest, although the early and later history of the germ cells in these insects is not known. In the diving beetle, *Dytiscus marginalis*,²³ a single oogonium gives rise to fifteen nurse cells and one oocyte. The oocyte and its mother cell, grandmother cell, and great-grandmother cell can be dis-

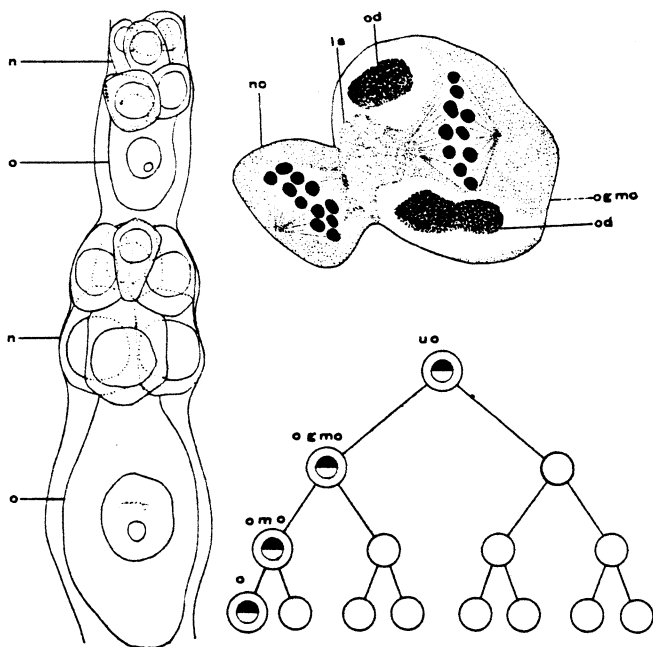


FIG. 9. Diagrams illustrating differential divisions during the formation of nurse cells in the whirligig beetle, *Dineutes nigror*. (Hegner and Russell, 1916.)

a. Part of the growth zone of an ovarian tubule showing two oocytes (o) each accompanied by seven nurse cells (n).

b. Division of two-cell stage. An ultimate oogonium has divided forming a nurse cell (nc) and an oocyte grandmother cell (ogmc) containing the oocyte determinant. is = intercellular strand.

c. Diagrammatic representation of oocyte differentiation. The plain circles indicate nurse cells. uo = ultimate oogonium containing the oocyte determinant within its nucleus. ogmc = oocyte grandmother cell. o = oocyte.

²³ Giardina, 1901, *Internat. Monatssch. f. Anat. u. Phys.*, Bd. 18; Debaisieux, 1909, *La Cellule*, T. 25; Gunther, 1910, *Zool. Jahrb.*, Bd. 30.

tinguished from the nurse cells by the presence of a peculiar ring of nuclear material within the cytoplasm and by their larger size. The gyrinid beetle, *Dineutes nigrior* (Fig. 9), resembles *Dytiscus* in general, but the ultimate oogonium passes through one less division, thus giving rise to one oocyte and only seven nurse cells.²⁴ The important fact is that during these differential divisions, in both cases, the nurse cells, which may be considered somatic since they are unable to reproduce, are deprived of part of their nuclear material. Apparently they differ from their sister cell, the oocyte, in this one respect, and it is therefore the presence of this nuclear material that makes it possible for the oocyte to develop into a new individual. This is one of the most striking cases of the passage of large masses of nuclear material into the cytoplasm. No such differential divisions have been discovered in chrysomelid beetles nor in the other insects where the nurse cells arise from oogonia, but they may occur in some way that has not been revealed by our methods of research.

The writer has discussed this subject rather fully with relation to the origin of nurse cells and oocytes in the honeybee.²⁵ In this insect a single oogonium gives rise to a rosette-like group of cells that are connected with one another by strands—probably of a mitochondrial nature—the remains of preceding mitotic divisions. There is no visible difference among the cells in a rosette which are hence apparently potentially alike. Nevertheless one or several from each rosette enlarge to form oocytes which are nourished by the rest acting as nurse cells. What determines the differentiation of certain cells into oocytes is not known but the following hypotheses have been expressed.

Three explanations have occurred to me: (1) There may be differential changes during the mitotic divisions in rosette formation as in *Dytiscus* resulting in one or more cells (oocytes) which differ in con-

²⁴ Hegner and Russell, 1916, *Proc. Nat. Acad. Sc.*, Vol. 2.

²⁵ Hegner, 1915, *Journ. Morph.*, Vol. 26.

tent from the others (nurse cells). No visible changes of this sort were observed. (2) The polarity of the rosettes may influence the cells in such a way that those near the center of the ovariole and closest to the zone of differentiation tend to develop into oocytes. (3) Those cells of the rosettes which reach the zone of differentiation first are stimulated to become oocytes and by their growth and differentiation prevent the other cells of the rosettes from similar changes.

4. Constitution of the Oocyte at the Beginning of the Growth Period

Very soon after the nurse cells are formed and the oocytes begin to enlarge the main axis of the oocyte in all

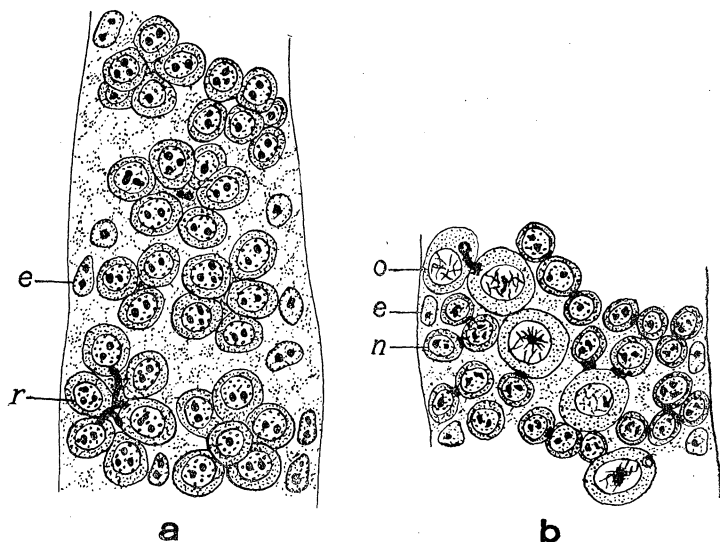


FIG. 10. The formation of oocytes in the honeybee. (Hegner, 1915.)

a. Part of an ovariole showing the rosettes (*r*) each resulting from the division of a single oogonium.

b. Part of an ovariole in the zone of differentiation showing five oocytes (*o*), many nurse cells (*n*) and epithelial cells (*e*).

insects seems to be established. The germinal vesicle at the same time changes its position from the center of the cell to a point near the nurse-cell chamber at the anterior end as described above in *Miastor* (Fig. 8). At what stage bilateral symmetry becomes fixed has not been determined.

The oocytes of insects at the beginning of the growth

period differ from the other cells in the body in the following ways. (1) In all cases where germ-line determinants occur the oocytes alone are provided with them and with the cytoplasm in which they are embedded. (2) In insects like *Miastor* a full amount of chromatin is present only in the oocytes. (3) In *Dytiscus*, *Dineutes* and probably other insects the oocytes contain nuclear material of which the nurse cells are deprived, but this may be interpreted simply as a means of inhibiting the reproduction of the latter and of changing them into nurse cells. (4) The oocytes seem to have no influence upon the development of the individual in which they lie, as indicated by castration and transplantation experiments,²⁶ and are in a comparatively undifferentiated condition when the growth period begins.

²⁶ Meisenheimer, 1912, Fests. 60 Geburtstag von Dr. J. W. Spengel III.; Kopec, 1911, *Arch. Entev.-mech.*, Bd. 33.

(To be continued)